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CARBON DIOXIDE BIOFIXATION BY *Chlorella* sp. IN A BUBBLE COLUMN REACTOR AT DIFFERENT FLOW RATES AND CO_2 CONCENTRATIONS

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Abstract - CO_2 biofixation of the microalgae *Chlorella* sp. for different CO_2 concentrations and gas flow rates in a bubble column reactor has been investigated in this study. Microalgae were cultivated under different CO_2 concentrations (at 1.75% and 9.45% v/v) and gas flow rates (at 30, 50 and 70 ml/min). The maximum specific growth rate of *Chlorella* sp. was obtained for the CO_2 concentration of 1.75 % and the gas flow rate of 50 mL/min. The highest biomass productivity rate (at 0.17 g L^{-1} day^{-1}) was for a sample with 1.75 % CO_2 at a flow rate of 70 ml/min. Moreover, the results have shown that the specific growth rate and CO_2 biofixation have a direct relation with culturing of *Chlorella* sp. Also, limiting CO_2 supplementation noticeably decreased biomass concentration. Therefore, the results have shown that a high flow rate and low concentration of CO_2 might promote a decrease in CO_2 fixation efficiency by *Chlorella* sp.
Keywords: CO_2 biofixation; Greenhouse gas; Microalgae; Biomass production; CO_2 concentration.

INTRODUCTION

Climate change is considered to be an important issue for the environment and ecosystem. This phenomenon becomes far worse when more carbon dioxide (CO_2) is emitted into the atmospheric air (Schneider, 1989; Yen et al., 2015). According to some research, more carbon dioxide will be produced due to growing manufacturing industry, forms of transport and human activity (Belbute and Pereira, 2015; Monastersky). Despite the fact that the utilization of fossil fuels (oil and coal) is the decisive factor in global warming and climate change, the widespread use of an alternative renewable source of energies can play an important role in reducing environmental pollution and preventing using non-renewable resources, which are quite limited in the world (Gharabaghi et al., 2015; Amin, 2009; McKendry, 2002; Sawayama et al., 1999).

Microalgae are known as the third generation of renewable biomass resources for biofuel and bio-based chemical production (Lee and Lavoie, 2013). In addition, microalgae have been described as a biological technology for CO_2 capture because of their high ability of photosynthesis (Amin, 2009; Cuéllar-Franca and Azapagic, 2015; Martínez et al., 2013). Microalgae are mostly cultivated under atmospheric air (containing 0.03% of CO_2). They can also grow in high levels of CO_2 such as Flue gas from power plants (containing 12%–15% of CO_2). Several studies have investigated microalgae cultivation under CO_2-supplementation (Thomas et al., 2016; Watanabe and Fujii, 2016). Utilization of CO_2 for microalgae cultivation has some advantages such as low cost, direct CO_2 capturing from exhaust gas and simplicity in operation. Some researches confirm that microalgae are capable of biofixating 10-50 times more carbon

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dioxide than terrestrial plants (Li et al., 2008; Rosenberg et al., 2011; Soares et al., 2013).

Additional CO_2 is necessary for the cultivation of microalgae through the photosynthetic process. However, a high carbon dioxide level in the medium of culture will cause a drop in the pH value, which inhibits the microalgae growth. Furthermore, microalgae cultivation under limitation in carbon dioxide usually restricts growth (de Godos et al., 2014; Yen et al., 2015). There are some parameters for optimization of CO_2 biofixation depending on the species of microalgae, such as the nutrient availability, light intensity, temperature, pH, sufficiently high surface area to volume ratio (height to diameter ratio), and countercurrent flow of gas and liquid at optimal flow rates (Cheng et al., 2013; Di Iaconi et al., 2006; Jacob-Lopes et al., 2008; Janssen et al., 2003; Mohsenpour and Willoughby, 2016). Among microalgae species, *Chlorella* species are considered as a suitable choice for carbon dioxide fixation. In addition, *Chlorella* sp. have been highlighted because of their outstanding ability for converting high levels of CO_2 in the input air of a photobioreactor into biomass (Chiu et al., 2008; Maeda et al., 1995).

In order to obtain the best operational condition for CO_2 removal, the cultivation of *Chlorella* sp. was investigated under different CO_2 concentrations and gas flow rates. Accumulation of fixed CO_2 and daily CO_2 fixation rate were also obtained for different conditions.

MATERIALS AND METHODS

Microalgae and culture medium

A culture of *Chlorella* sp. (PTCC 6010, Persian Type Culture Collection) was obtained from the Iranian Research Organization for Science and Technology (IROST) (Tehran, Iran). The cells of *Chlorella* sp. were cultured in Rudic's Medium (Delavari Amrei et al., 2015) (per liter), including 33 g Sea salt, 20 mg NaCl, 10 mg $MgSO_4.7H_2O$, 47 mg $CaCl_2$, 300 mg $NaNO_3$, 20 mg KH_2PO_4,80 mg K_2HPO_4, 0.1 mg $ZnSO_4.7H_2O$, 7.5 mg Na_2.EDTA, 17 mg $FeCl_3$.$6H_2O$, 0.3 H_3BO_3, 0.3 mg $(NH_4)_6Mo_7O_{24}.4H_2O$, 17 mg $FeCl_3.6H_2O$, 0.2 mg $Co(NO_3)_2.H_2O$, 1.5 mg $MnSO_4.H_2O$, 0.08 mg $CuSO_4.5H_2O$, 0.1 mg $ZnSO_4$.$7H_2O$.

Experimental setup and cultivation conditions

The microalgae were cultured in a cylindrical glass reactor (diameter 14 cm, height 1 m) with 10 L of working volume. Cultures were placed on a bench at 26 ± 1 °C under cool-white fluorescent light (General Electric) for 16 days. Light intensity was approximately 87.75 µmol m^{-2} s^{-1} at the surface of the photobioreactor, measured with a Lux-meter (HTC, India; Model no. 102). The experimental set-up is presented in Fig. 1.

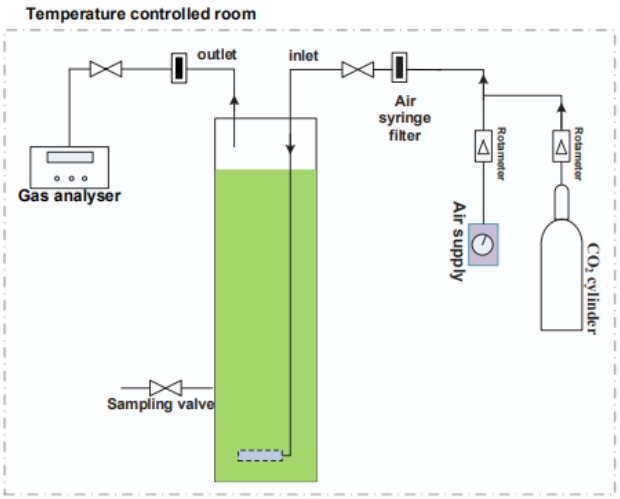

Figure 1. Experimental set-up.

Ambient air was mixed with CO_2 to give optional concentrations by a gas mixer instrument. Aeration gas was filter sterilized and pumped into the microalgae culture medium. The CO_2 and O_2 concentrations of the gas in the input and output of the photobioreactor were measured using a STAR GAS global diagnostics system. A portable multiparameter (pH/DO/Temp/EC) HANNA HI98194 instrument was used in each run of photobioreactor to monitor online and record dissolved oxygen (DO), temperature, and pH. In each case, the probes were put in the photobioreactor for 2 minutes. All the experiments were repeated in triplicate, and the average values were represented.

In this study, four runs of experiments for microalgae growth under various CO_2 concentration levels and gas flow rate were applied (Table 1).

Table 1. CO_2 concentration and gas flow rate for different runs.

Exp. No.	CO_2 concentration (v/v %)	Gas flow rate (mL/min)
Run 1	9.45	50
Run 2	1.75	50
Run 3	1.75	30
Run 4	1.75	70

Growth monitoring and assessed parameters

The optical density of broth was measured by the absorbance at 560 nm in an Ultrospec 3300 pro UV/Visible spectrophotometer (Amersham Biosciences, Cambridge, UK). The relationship between the biomass concentration (X, g/L) or dry weight and optical density (OD_{560}) is obtained as follows (Delavari Amrei et al., 2014):

$$X = 0.49 \times OD_{560} \quad (1)$$

Various concentrations of carbon dioxide were utilized for aeration of the culture medium inside the

photobioreactors. Air containing 0.03% CO_2, 1.75% and 9.45% CO_2 was used to evaluate the effect of improving CO_2 fixation rates. The selected proportion of CO_2 in these aeration conditions was based on the composition of CO_2 in industrial flue gases.

Based on the mass balance of microalgae, the fixation rate of CO_2 can be evaluated. The accumulation of fixed CO_2 (FA, g CO_2) was calculated based on Eq. 2 (Moraes et al., 2016):

$$FA = (X_t - X_0) \times C_c \times V \times \left(\frac{M_{CO_2}}{M_c} \right) \quad (2)$$

where X_t ($g.L^{-1}$) is the concentration of the biomass at time t (days), X_0 ($g.L^{-1}$) is the concentration of microalgae at time t_0, C_c (g_c/g_{sample}) is the fraction of carbon determined in the biomass, V (L) is the volume of the photobioreactor, and M_{CO2} and M_C ($gmol^{-1}$) are the molar masses of carbon dioxide and carbon present in the microalgae biomasses, respectively. Carbon dioxide is the main source of the carbon content of the microalgae cell. A mole of CO_2 has a mass of 44 g including 12 g of carbon. The daily CO_2 fixation rate (FD, $g_{CO2BioFixed}/g_{CO2InjectedDay}$) was achieved during the growth period according to Eq. 3:

$$FD = \frac{(FA_{(t+1)} - FA_{(t)})}{m_{id}} \quad (3)$$

where $FA_{(t+1)}$ and $FA_{(t)}$ are the CO_2 accumulated at time t+1 (day) and time t (day), respectively. Also, m_{id} (g_{CO2}) is the mass of daily injected of CO_2. Therefore, the maximum daily CO_2 fixation rate (F_{CO2}) was achieved at the maximal (FD) value during the growth period.

The specific growth rate (μ) is calculated according to the following equation. Substantially, the specific growth rate is the slope of the biomass productivity curve or, in other words, the daily rate of the biomass growth.

$$\mu = \frac{\ln\left(\frac{C_n}{C_{n-1}} \right)}{t_n - t_{n-1}} \quad (4)$$

where C_n and C_{n-1} are the biomass concentrations (g L^{-1}) on the days t_n and t_{n-1}, respectively.

RESULTS AND DISCUSSION

Effect of different concentrations of CO_2 on microalgae culture

Fig. 2 presents the growth curves obtained for *Chlorella* sp. grown in air streams containing the different CO_2 concentrations and different flow rates that are presented in Table 1. Also, the pH variation of samples is shown in Fig. 3. The average range of pH is between 7 and 8. In the first run of experiments, the culture of microalgae was aerated after four days with 9.75 % CO_2 and a gas flow rate of 50 mL/min. The maximum growth of microalgae happened in the fifth and sixth days and the final dry cell concentrations were 2.15 g/L after 16 days.

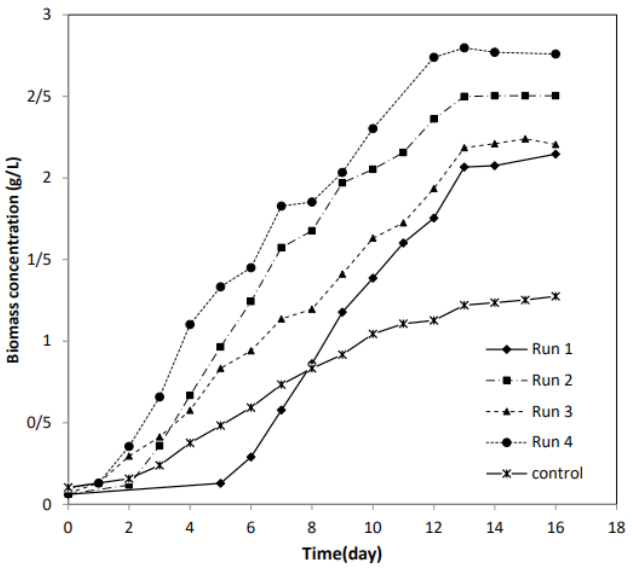

Figure 2. Time course for cell growth at different concentrations of CO_2.

As shown in Figs. 4 and 5, the maximum level of CO_2 removal occurred on the day that μ was the maximum for all of the experiments. In fact, for the first run n the 6th day, the microalgae growth rate was the highest, at 0.81 1/day, and more CO_2 was consumed so that the maximum of daily CO_2 biofixation rate was

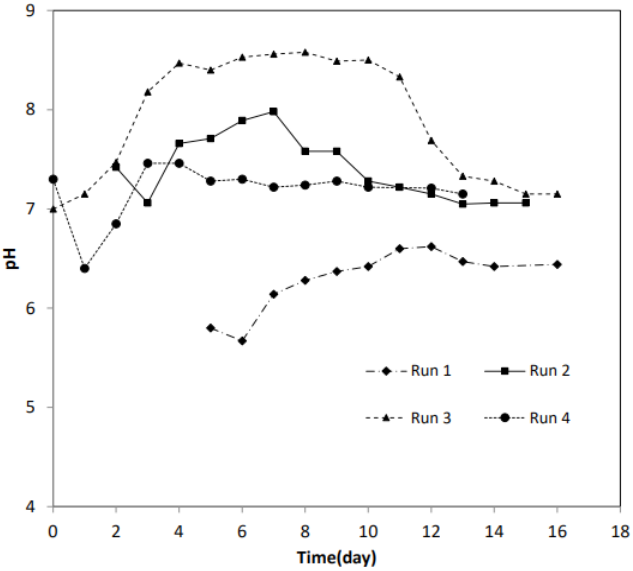

Figure 3. pH variations of the microalgae under different CO_2 aeration levels.

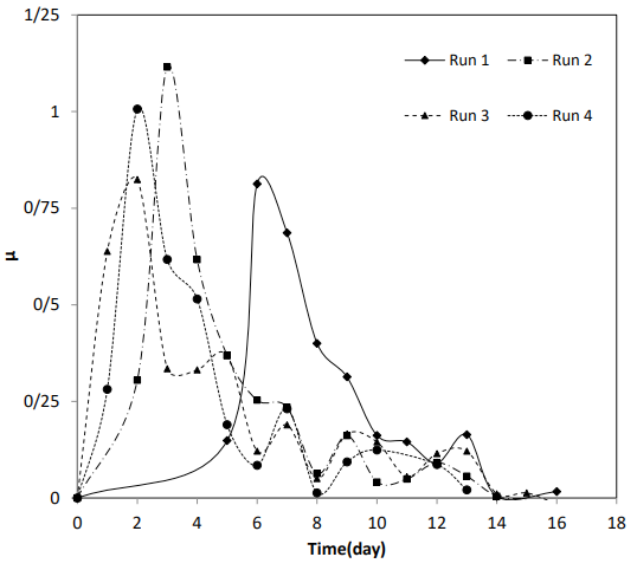

Figure 4. Specific growth rate variations of the microalgae under different CO_2 aeration levels.

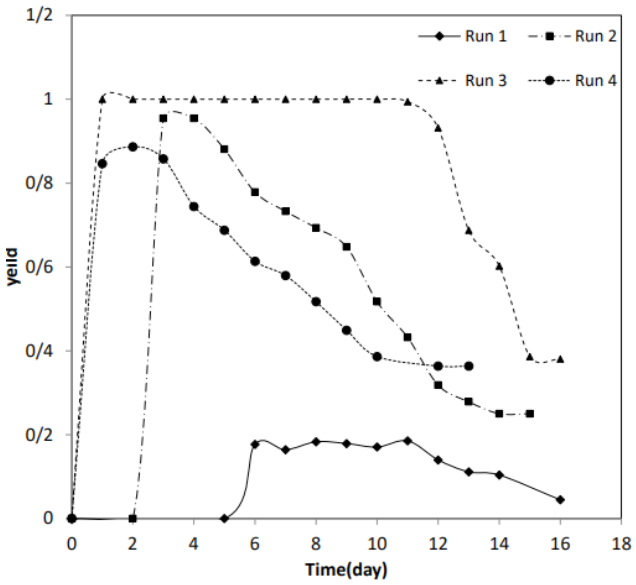

Figure 5. CO_2 removal percent for different CO_2 aeration levels.

about 17%. Therefore, the μ value and the percentage of CO_2 removal are closely linked. In the first run, there was a long lag phase due to high CO_2 concentration (at 9.45 %). Furthermore, the exponential phase, as well as the maximum level of CO_2 removal, occurred on the sixth day. Also, in the first run, with increasing mass concentration in the photobioreactor and turbidity of the solution after the eleventh day, the percentage of CO_2 in the output gas climbed by about 5.6% (from 7.7 to 8.13).

CO_2 concentration has an impact on the metabolism of carbon and algae photochemical properties (Zhao and Su, 2014). Because carbon dioxide is the main source of carbon, a limited supply of carbon dioxide concentration will inhibit algae productivity. A high CO_2 concentration could inhibit algae growth because of a decrease in pH to lower than 5.5 in the cultivation system.

In the second run of experiments, the solution of microalgae was aerated after two days with a CO_2 concentration 1.75 % and flow of 50 mL/min. Dry mass of algae in the second run reached its highest value at 2.5 g/L on the 12th day. The dry mass of microalgae increased in comparison with the first run due to lower CO_2 concentration and a rise in the average pH from around 6.3 to 7.5. On the third day, the μ value peaked at 1.1 (day^{-1}).

In the third run of experiments, for investigating the effect of low gas flow rate and limited CO_2 access, the solution of microalgae was aerated with 1.75 % CO_2 and a low flow rate 30 mL/min. The dry mass concentration of algae fell to 2.25 g/L on the 16th day (10% decrease in comparison with the second run) due to low CO_2 flow rate. The main cause is the lack of sufficient carbon dioxide that is considered as the main carbon source.

High mixing intensity could harm algae, while a sufficient mixing intensity is required for transferring of substances. In the fourth run of experiments, for the investigation of the effect of the high gas flow rate, the solution of microalgae was aerated with 1.75 % CO_2 and a flow rate of 70 ml/min.

As shown in Fig. 4, as in the second and third runs, in the fourth run, the μ value on the second day after aeration reached its highest value. Also, the maximum level of CO_2 fixation yield on the second day for the second, third, and fourth runs happened at 100 %, 97%, and 94%, respectively. The plus point of this test was that the dry mass concentration value peaked at 2.8 g/L in 13 days in comparison with the other runs due to proper mixing in the system and an adjusted pH value with the microalgae activity in the optimal condition.

The pH of the algae culture is related to the solubility and availability of carbon dioxide in the photobioreactor (Mohsenpour and Willoughby, 2016). Bicarbonate is the determining factor in the photosynthesis of microalgae. Variation of the pH results in changing proportions of carbon compounds (carbon dioxide, bicarbonate, and carbonate). Nevertheless, among these compounds, the consumption of carbonate in algae photosynthesis is still being investigated and discovered (Price et al., 2004). Therefore, the amounts of carbon dioxide and bicarbonate transporters may be the main contributing factor in determining the carbon transfer rate. It should also be noted that the carbon transfer rate may make changes in the ratios of assimilation of carbon and nitrogen and affect the cell composition (Hsueh et al., 2007). When the pH

of the algae cultivation is between 6.8 and 8.4, more than 50% of the inlet CO_2 converts to bicarbonate and, at pH 8.4, the carbon dioxide converts to bicarbonate completely. So, the optimal condition for algae growth will be achieved in the range of approximately pH 6.8 to pH 8. Similarly, Hsueh et al. (2007) have shown that, whereas the maximum growth rate was achieved in approximately the above -mentioned range, this rate decreased at the higher pH. Thus, it was logical that the maximum dry mass concentration was obtained in the fourth run and the photosynthesis increased in this run.

As shown in Fig. 3, in the first run, the pH value of the solution rose exponentially (until the eleventh day) from 5.8 to 6.62 and declined steadily to 6.4 with decreasing microorganism activity.

In the second and fourth runs, with 1.75% CO_2 concentration, the initial value near neutral pH remained steady at 7.5 and 7.3 for a gas flow rate of 50 and 70 ml/min, respectively. There was a slight increase in the pH during the aeration flow of 50 and 70 mL/min; however, in the third run, there was an obvious increase in the pH and it remained steady at 8.5 because the amount of CO_2 in the solution was lower. This may be attributed to the relation between pH, biomass growth and photosynthetic demand. With increasing CO_2 in the algal solution, the pH will decline. On the other hand, with rising CO_2, photosynthesis and algae growth will rise and algae growth leads to an increase in the pH. The same results have been achieved by several authors. According to de Morais and Costa (2007) and Grobbelaar (2004), the photosynthetic process of CO_2 fixation, owing to the accumulation of OH^-, leads to a moderate rise in pH. On the other hand, the dissolution of CO_2 in water brings about acidification because of the formation of carbonic acid. Moreover, Chinnasamy et al. (2009) also observed that the initial pH decreased with increasing CO_2 concentration and consequently the pH increased with the growth of *C. vulgaris*.

As shown in Fig. 4, the specific growth rate of microalgae in the minimum of the concentration of CO_2 (at 1.75%) is more than at the maximum of the CO_2 concentration (at 9.45%). This is because of a plummet in the pH value with increasing CO_2 concentration of the solution.

As shown in Fig. 2, the CO_2 supplement has a positive effect on the growth rate and the mass production of the microalgae in comparison with the absence of CO_2 gas aeration. As a result, without CO_2 the growth curve was linear (R^2=0.9145), while it changed with CO_2 aeration to an exponential shape. As shown in Figure 2, the maximum production of biomass was at 1.27 g/L in ambient air, which was significantly lower than biomass in culture under CO_2 supplementation.

Carbon fixation

The accumulation of fixed CO_2 (FA, g_{CO2}) by *Chlorella* sp., calculated using Equation (2) for the experimental runs, is reported in Table 2. The highest figure for the accumulation of fixed CO_2 of 49.5 g_{CO2} was seen at a CO_2 air ratio of 1.75% and 70 ml/min. With increasing flow at 1.75% CO2 air ratio, the daily CO_2 biofixation rate decreased due to the limited capacity of algae photosynthetic.

Also, as shown in Fig. 5, maximum daily CO_2 biofixation happened in the third run (at 1.75 % CO_2 air ratio and at 30 ml/min flow). In the third run, the CO_2 content in the output gas was roughly zero. Therefore, the maximum figure for removed carbon dioxide and produced oxygen can be easily calculated per gram of algae. The O_2 production and CO_2 consumption by the system per gram of algae is 0.603 $g_{O2}g^{-1}day^{-1}$ and 0.624 $g_{CO2}\,g^{-1}day^{-1}$, respectively. Furthermore, on the second day, the CO_2 biofixation rate and μ value peaked simultaneously, owing to the high algae absorption in the exponential growth. The CO_2 biofixation rate also remained at 100% as the algae entirely consumed the inlet CO_2 to fix it.

Table 2. Maximal biomass concentration (Xmax, g L^{-1}), maximal specific growth rate (μ_{max}, day^{-1}), maximal daily CO_2 biofixation rate (F_{CO2}, % v/v) obtained for the runs of the experiment.

Exp. No.	Xmax ($g.L^{-1}$)	μ_{max} (day^{-1})	Biomass Productivity Rate ($g\,L^{-1}\,day^{-1}$)	F_{CO2} (%)	FA (gCO_2)
Run 1	2.14	0.81	0.12	17.67	37.2
Run 2	2.5	1.11	0.15	95.45	44.7
Run 3	2.25	0.82	0.14	100	40.0
Run 4	2.8	1.00	0.17	88.63	49.5

CONCLUSIONS

This study reveals that culturing microalgae with different levels of CO_2 concentrations can lead to various microalgae specific growth rates. So, CO_2 biofixation is of major significance. The highest value of μ was found at 1.75% CO_2 and 50 mL/min flow rate in 1.11 day^{-1}. This paper shows that the best biofixation of CO_2 was 44.7 g_{CO2} after 16 days, which occurred in the region where μ was high and CO_2 accumulation was the maximum. At 1.75 % CO_2 air ratio and at 30 mL/min flow, 100% CO_2 biofixation occurred, but a significant and high efficiency of the growth rate was not observed due to the lack of carbon dioxide. As a result, the microalgae *Chlorella* sp. has a great potential for biofuel production and CO_2 capturing so as to reduce the negative impacts of greenhouse gas and global warming.

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